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INVESTIGATION AND UTILIZATION OF BEHAVIOURAL PRINCIPLES THAT INFLUENCE BEEF PRODUCTION

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Investigation and utilization of behavioural principles that influence beef production

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Investigation and utilization of behavioural principles that influence beef production. Joseph M. Stookey

Abstract/Summary: The overall objective of this project was to investigate basic principles that influence beef cattle behaviour and apply these principles to enhance or improve productivity. Several distinct areas under this "umbrella approach" were investigated. Previous observations on the mating behaviour of cattle led us to hypothesize that variation in breeding success could be partially related to females' preference for certain bulls. If true it would be useful to know the characteristics that females are avoiding or selecting. We conducted two studies to determine if female cattle demonstrate mate choice. In Exp. 1 when oestrous females were given free access to bulls confined behind partitions **we found that oestrous female cattle preferred to spend their time near a pen that housed other females, but not with bulls**. Because female cattle will form sexually active groups with other females to possibly attract males (which might explain their preference for a pen of females) we wondered whether confining the bulls behind partitions was interfering with a female's ability to interact and possibly "lure" her preferred bull to her. Therefore, in Exp 2, bulls were individually confined to pens by training them with shock collars to not exit the open gate. In this set up oestrous females were free to move in and out of bull pens without restrictions. Again, **oestrous females showed no real preference for a particular bull**. So even though mating success varies greatly between bulls in a multi-sire mating system we found no evidence that females are making active selections for a particular bull. In pasture conditions females are likely dependent upon the bulls to sort out dominance rank to gain access to them. Using GPS collars on bulls in a community pasture we tracked the movement of 11 bulls to see whether bulls occupied a home range or fixed territory (which if true might also explain the wide variation in breeding success). Only 4 collars yielded useable data, **but we found no evidence using GPS collars that bulls restricted themselves to certain areas within a pasture**.

We also investigated a novel way to deliver vaccines using plastic anti-sucking device that are used in the new Two-stage weaning procedure. Quietwean nose tags were modified by drilling a cavity into both nose tongs to hold either IBR or BVD vaccines suspended in a gel. One hundred calves were used and all had blood samples taken at the start of the trial to establish baseline titer values and again 14 days after vaccination. All calves were weaned following the two-stage weaning technique. Each vaccine was tested using two methods of delivery: either mixed in a gel and inserted into a cavity in the Quietwean tag or delivered following the recommended technique developed by the vaccine company, either intranasal (for TSV-2) or intramuscular injection (for Bovishield). In both cases **vaccines delivered via the Quietwean nose tags (intranasal) were as good as or better in launching a response than the same vaccine when administered in the recommended way.** Coupling Two-stage weaning with vaccine delivery has multiple advantages including timeliness and a needless delivery.

In an experiment using 40 heifers we demonstrated, using strain gauges attached to a headgate, that cattle make an association between their behaviour during restraint and the events that follow. **Releasing cattle from a headgate while they are struggling will, in effect, teach them to struggle during subsequent restraint. It would be prudent for handlers to wait for a period of calm during restraint prior to releasing them.**

Final Report:

Background:

Following are a series of studies that were undertaken to investigate and apply behavioural principles in beef production. The studies are diverse, but are connected in common to a behavioural theme. All the projects listed were funded in part or total by funds from this ADF grant (acknowledgements to other funding bodies are listed when appropriate). Some of the projects were attempts to understand at the basic level the motivation that may explain behaviours that we normally observe under field conditions (i.e. why is there wide variation in breeding success among bulls in a multi-sire mating system?). Other projects in this report attempt to apply basic principles to improve or enhance productivity (i.e. Use of the Two-stage weaning protocol to understand how stress influences the immune response and use of the plastic Two-stage weaning anti-sucking devices to deliver vaccines.) A final project is an attempt to take advantage of animals' ability to form associations between their behaviour and subsequent events (i.e. do cattle learn to associate their struggling during restraint with release and if so could we take advantage of that associative learning to make cattle calmer?).

Each individual project in the report is identified by a title and the names of the individuals who worked on the project. Each project has a background/introduction, methods, results, conclusions and recommendations (when appropriate). A couple of the projects "reports" are formatted as manuscripts that were (or will be shortly) submitted for publication.

Mate selection by female Bos Taurus beef cattle

R. A. Kendall, J. C. Petherick, J. M. Stookey, J. M. Watts, and D. Olenick

Abstract

Cattle are polygynous and extensive beef cattle enterprises frequently use multiple bulls in their breeding herds. Mating success and fertility of bulls within herds show significant individual variation that appears to be related to social relationships and sexual competition between bulls, but it is not clear whether female choice also plays a role in determining bull mating success. Two studies investigated mate selection by female *Bos taurus* beef cattle. In the first experiment oestrous females (n = 20) were individually tested in an arena containing three individually penned bulls and a pen containing two female group-mates. Preference was determined by the relative amounts of time spent in proximity to the four pens. Test females spent a significantly greater proportion of time near to the stimulus animals than away from them ($t = 6.61$; 19 d.f.; $P<0.001$) and significantly more time with their group-mates than the bull they spent most time near (35.8% v 13.6% SE = 4.9). Only four females spent more time with a bull than with their group-mates. There was evidence ($P<0.001$) that the proportion of time spent with bulls differed between bulls, indicating some preference, although different females preferred different bulls and the proportion of time spent near bulls was generally low. The second experiment, used aversive training with audio and electrical stimuli to confine bulls in open pens that were freely accessible by oestrous and non-oestrous heifers. Oestrous heifers did not display bull-seeking behaviour through increased entry rate into bullpens ($p=0.4175$), nor proportion of time spent within

bullpens (p=0.4503), relative to non-estrous heifers. Given the opportunity to select between pens containing bulls of differing age, and body weight, oestrous heifers did not exhibit mate preference as determined by number of entries into (Kruskal-Wallis Statistic=1.2425, p=0.5373), or duration of time spent within the pen of a particular bull (Kruskal-Wallis Statistic=3.1804, p=0.2039).

Introduction

Sexual selection theory holds that amongst polygynous animals in which females invest considerably more in offspring than do males, males should attempt to mate with as many females as possible, whereas females should exhibit mate choice (Trivers, 1972). Mate choice is defined as "any pattern of behaviour shown by members of one sex, that leads to their being more likely to mate with certain members of the opposite sex than with others" (Halliday, 1983). Female choice may involve preferential mating based on various male attributes including social status, sexual competence, quality of territory or resources held, physical condition, parental ability and/or effective courtship displays (Trivers, 1972). In many taxa in which males establish dominance relationships with one another, females have been shown to mate preferentially with a male of high status (Qvarnstrom and Forsgren, 1998).

Within a herd, cattle interact and develop relationships, including relationships that are of a dominant-subordinate nature, resulting in the formation of a 'dominance hierarchy' (Hafez and Bouissou, 1975; Fraser, 1985; Houpt, 1991). Bulls maintain a hierarchy through displays with little overt aggression (Houpt, 1991), wherein social status is positively correlated with age, seniority or length of time in the herd, and body weight (Rupp et al., 1977; Blockey, 1979).

Most of the literature on the sexual behaviour of cattle is largely descriptive in nature. The emphasis is on bull behaviour and libido (for a recent review, see Petherick, 2005), with the role of the female being largely ignored. Previous work has demonstrated that the mating success of bulls differs significantly between individuals. In particular, there is evidence that dominant bulls perform more services than do subordinates (Rupp et al., 1977; Blockey, 1979; Fritz et al., 1999), but it is not clear what roles the processes of male competition and female choice play in the determination of mating success. The presence of a dominant male has been shown to inhibit and/or interfere with the sexual behaviour of subordinate males, (see Rupp et al., 1977; Blockey, 1979; Price, 1987; Crichton and Lishman, 1988; Lopez et al., 1999), which suggests that competition and social relationships between males play a significant role in determining mating success. However, the existence of intense intra-male competition does not preclude the expression of female choice (Halliday, 1983). In fact, there is evidence suggesting that estrous cows may exhibit bull-seeking behaviour (Fraser, 1985), and that they may demonstrate a preference for dominant bulls. For example, Blockey (1979) reported that estrous heifers actively solicited attention from bulls by following, licking and occasionally mounting them and that 82% of such activities were directed to the dominant bull. Similarly, in his research on American bison, Wolff (1998) concluded that

females used several behavioural strategies to solicit male attention, to incite male-male competition, and ultimately to acquire dominant bulls as mates.

We investigated whether estrous cattle demonstrate bull-seeking and mate selection in two experiments. In the first, the stimulus animals were confined to pens and could not be accessed by the test female. In the second experiment, access to bulls by the females was made possible by using aversive training with audio and electrical stimuli to confine bulls in open pens. We examined whether female beef cattle exhibited preference by recording the location of the test females and the amount of time spent with bulls of differing age and body weight. Confining the bulls prevented males from sequestering females, and eliminated direct intra-male competition, which could otherwise confound or obscure female preferences. We hypothesized that oestrous females would demonstrate preference by spending different amounts of time in proximity to and interacting with different bulls and, in comparison to non-oestrous females, would show bull-seeking behaviour by entering the pens more frequently and for longer durations. We predicted that females would prefer an older, larger bull as this was likely to reflect social status.

Materials and Methods

Location and animals

The studies took place at the University of Saskatchewan's Goodale Farm (Saskatoon, SK 52° 10' N 106° 41' W) during summer months, May to July. The beef herd was managed according to guidelines set by the Canadian Council on Animal Care (1993). Experimental protocols were approved by the University of Saskatchewan's Animal Care Protocol Review Committee (UCACS # 20030034 and #20050042).

Large rectangular enclosures containing the individual pens were used as the test arenas. In experiment 1, the arena measured approximately 81.4 m x 27.4 m and four individual pens measuring approximately 6.1 m x 3.05 m were constructed along one side (Fig. 1). For experiment 2, the arena measured approximately 29.2m x 27.7m and contained three individual bull pens (9.6m x 10.7m) along one side (Fig. 2).

The pens within the arenas contained the stimulus animals; in experiment 1 these comprised individually-penned bulls in three pens and two group-mates of the test female in the fourth pen. The location of the test animals was randomly allocated for each test female. In experiment 2, each of the three pens contained a bull. The test females were individually introduced into the arena and were free to range throughout the enclosure. In experiment 1 the test females could not enter the pens of the stimulus animals, but in experiment 2, they could enter the bull pens. The bulls in these pens were trained not to exit using Sportsman^{R} (Tri-tronics, USA) remote trainers attached to their halters (see below).

Females were classified as being in oestrus when they displayed behavioural signs including: restlessness; walking excessively; standing close to other cows, exhibiting chin

resting and/or attempted mounts; tail or vulva sniffing; and frequent urination (Albright and Arave, 1997). True oestrus is characterized by a cow being willing for other cattle, male or female, to mount her (Phillips, 2002); therefore this was used as the primary indicator for classification. Females not displaying behavioural signs of oestrus were classified as non-oestrous.

Experiment 1

The test females were three Hereford heifers and 17 Hereford cows all of which were observed to be in standing oestrus at the time of testing.

During the test, 11 of the cows had a calf with them and one had twins. The remaining five cows had been hormonally stimulated to super-ovulate for other experimental work not associated with this experiment. Outside of the testing time, the cows were grouped with other cows and calves in an enclosure opposite the test arena and separated by a road. The heifers were grouped with other heifers in an enclosure adjacent to that housing the cows and calves.

The stimulus bulls were Hereford, two were 2 years old and weighed 832 kg (number 70) and 766 kg (number 57), and the third was 3 years old and weighed 937 kg (number 27). Outside of the testing time, the bulls were grouped together and with other bulls in a pen 250 m from the test arena. The group-mates were females with or without calves and were selected on the basis that they were showing no behavioural signs of being in oestrous.

Experiment 2

Three adult Hereford bulls and 20 heifers of various breeds and crossbreeds, varying in weight from 445 to 683 kg were used. The bulls differed in age and body weight: Bull 1 was a yearling (445 kg), Bull 2 was 2 years of age (557 kg), and Bull 3 was 5 years of age (683 kg). The heifers were individually marked with numbered ear tags. Overnight, the cattle were housed in pens other than the test enclosure; the bulls were housed in individual pens, whereas the heifers were group housed.

Experimental procedures

Experiment 1

Pre-exposure

Prior to the experiment, the bulls were given experience on four occasions of being moved to and from the test arena, confined in the pens for up to five hours and moved between pens. We felt it unnecessary to give the stimulus females experience, as they were penned with a group-mate and would be less motivated to try to escape from the pen compared to the bulls.

Testing procedure

During the tests hay and water were available in the pens of the stimulus animals, but animals also had feed and water available in their home enclosures.

The test arena was divided into a number of sectors by paint-marking fence posts and the ground (see Fig. 1) so that observers could record the location of the test cow continuously. Two observers recorded each animal, each person taking one half of the pen (Fig. 1).

For each test the position of the bulls and group-mates were randomly allocated and the animals placed in those pens. The test female (and calf) was introduced to the arena and recording started immediately with the female's location being recorded for one hour. She was then removed to her home enclosure and if another female was to be tested, then the bulls and group-mates moved to their new pens.

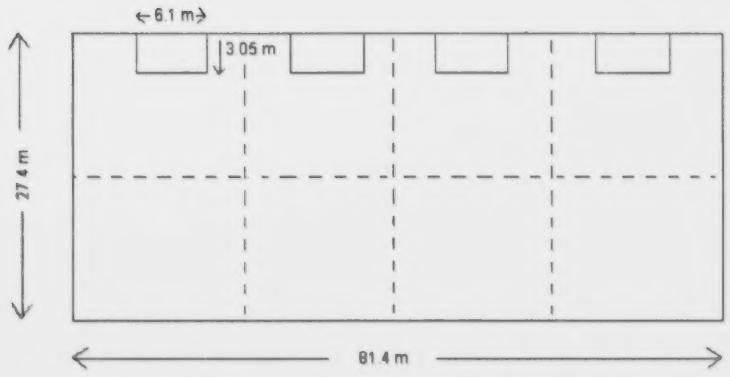

Figure 1. Diagram of pen layout. The four smaller rectangles represent the area where stimulus animals were kept and randomly distributed (3 bull pens and 1 stimulus group pen). Each test animal was free to roam throughout the rest of the enclosure. The dash lines represent the arbitrary areas that a test animal could occupy during observations, such that a test animal occupying a dashed rectangle near a stimulus pen was interpreted to mean a test animal was "near" a stimulus pen.

Experiment 2

Oestrus synchronization

For an unrelated experiment, 40 heifers were synchronized using progesterone inserts (EAZI-BREED^{TM}, CIDR^{R}, Pfizer) and 2 injections of a PGF2$\propto$ analogue, cloprostenol sodium (Estrumate^{R}, Schering Plough) and then artificially inseminated. Twenty heifers were determined not to be pregnant and were used for the female choice experiment. Of the study heifers, ten were again synchronized using PGF2$\propto$ (Estrumate^{R}); of these, seven would be expected to come into estrus since a scheme of two injections of Estrumate^{R} is believed to be 70% effective in synchronizing estrus

(Thompson, 1995). Each treatment heifer received 2ml (526 mcg) of PGf2∝ administrated intramuscularly. PGF2∝ administration was scheduled over a four day period so that 2-3 heifers received PG2∝ on each of days 11 through 15 of their estrous cycle, and could be then expected to come into estrus 72-96 hours later. They were observed on days 3 and 4 post-injection for the female choice experiment.

Pre-exposure

The bulls were exposed to the bull pens with the access gates closed and no heifers present on two occasions, for approximately 2 hours each time. Similarly, the heifers were exposed to the test enclosure and allowed free access to the bull pens for a single 2 hour period without bulls present. Three days prior to the beginning of the female choice experiment, the heifers were released, along with the bulls, into a pen other than the test enclosure for a single period of 2 hours. This pre-exposure allowed the heifers to associate with, and to potentially assess behavioural and physiological differences between the bulls prior to coming into estrus.

Bull Training

An initial attempt was made to tether the bulls, using a methodology similar to that used by Gibson and Jewell (1982) to restrain rams in an investigation of mate preference in domestic sheep. However, the bulls did not tolerate the tethers, and there appeared to be a serious risk of entanglement and injury, therefore we opted to train the bulls using Sportsman^{R} (Tri-tronics, USA) remote trainers designed for dogs. Each training unit consisted of a hand-held transmitter and a receiver mounted on a halter with two contact points that emitted electrical stimuli. Each training unit operated on a separate frequency, allowing the operator to individually control each receiver. The receiver emitted an audio signal when one button was pushed on the remote transmitter and an electrical stimulation when a second button was pushed. The electrical stimulation could be varied through five levels of increasing strength of intensity (with level five indicating maximal intensity), by changing the contact points on the receivers. For the two larger bulls, level four (yellow) contact points were used, whereas for the yearling bull, level three (orange) contact points were used (Tri-tronics, 2005). The halter was fitted with the receiver so that its contact points were positioned across the bridge of the bull's nose; the halter was adjusted each day during training and testing to ensure adequate contact of the points with the bull's skin. The halters were removed from the bulls each day at the end of the session.

Training of the bulls occurred over a period of four days, with each training session lasting 1-2 hours. The bulls were run through a chute to fit their halters, and then moved into their individual pens through gates at the back of their pen. At the access gates at the front of the bull pens, two half concentric circles of radii (m) were painted on the ground centered between the gate posts, extending back into the bullpen. The paint lines represented a visible threshold to the bull and to the remote trainer operator. The bull received an audio signal if he stepped over the first line and if he stepped over the second line, he received an electrical stimulation intended to stop or turn him prior to

exiting the pen.

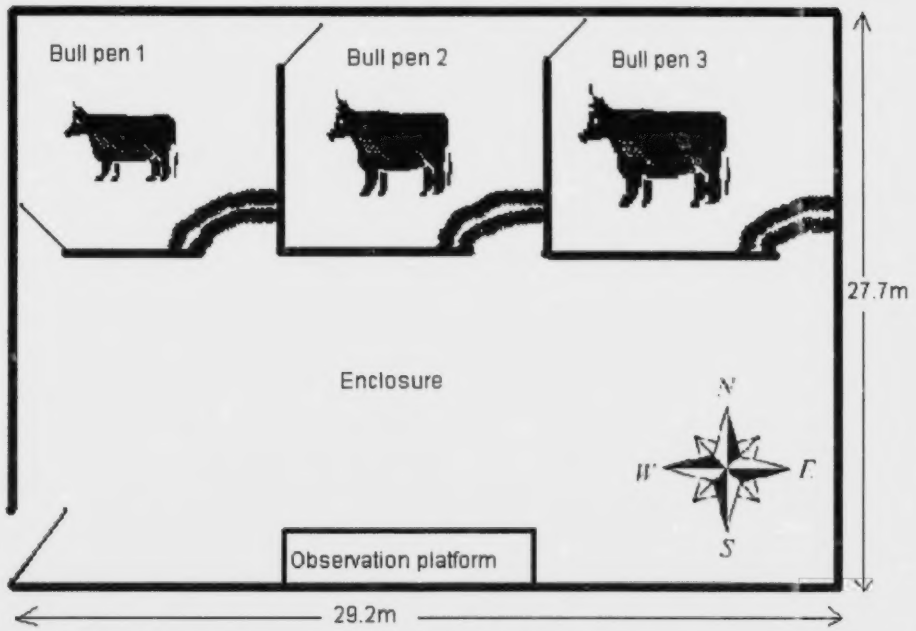

Figure 2. Pen layout. The pens are shown with the access gates open. The gates (shown in open position) at the south-western corner of the enclosure, the southwestern corner of pen 1, and the northeastern corners of pen 1 and 2 were used to bring the bulls and heifers into the test arena. These gates were closed during the female choice experiment. For the purposes of illustration, Bulls 1, 2, and 3 are shown in Pens 1, 2, and 3, respectively; in practice, the pen location of each bull was rotated each observation period.

On the first two days of training, the bulls were trained in their individual pens without the presence of heifers. On the first day of training, the opening of the access gate was initially restricted to a width of 1 m. Once each bull had received 1 or 2 shocks at the opening, the access gates were opened to the maximal width of 2.5 m. On the second day, the bulls were trained with the access gates again open to the maximal width. On the third and fourth days of training, the access gates were open to the maximal width and non-estrous heifers were brought into the enclosure and were allowed free access to the bull pens.

Testing procedure

Following the four training days, the female choice experiment was initiated. At the start of each observation period, 8-12 heifers (equal numbers of synchronized and non-synchronized heifers) were released into the enclosure and allowed to move freely throughout and to enter and exit the bull pens through the access gates. Prior to data collection, synchronized heifers were categorized as estrous or non-estrous based on behavioural parameters described above.

Because location and other characteristics (e.g. shade availability) of the individual bull pens could potentially influence heifer preferences, the location of each bull was changed randomly between pens for each observation period. Bulls were prevented from exiting their individual pens according to the training protocol wherein a bull received an audio signal if he crossed the first white line and an electrical stimulation if he crossed the second line.

The animals were observed continuously during daylight for a period of four to seven hours each day, depending on activity, from a platform 1.5 m above ground level located 22.4 m from the midpoint of the centre bull pen. Activity within the bull pens was also monitored by time lapse video (60 frames/s) using three cameras (Panasonic black and white camera WV-BL200) mounted on the north side of the enclosure, 2.6m above the ground, each of which viewed the field of movement of one bull. Video data was recorded with a screen splitter (Panasonic Quad System WJ-MS424) and video recorder (Panasonic time-lapse VCR AG-6730P). The following data were recorded:

1) All entries by heifers. A heifer was considered to have entered the pen if the point of her shoulder passed through the access gate.
2) Mounting behaviours displayed between a bull and heifer within a bull pen, including attempted mounts by the bull; mounts with the heifer standing for the bull; and mounts with intromission and ejaculation.

Scan samples were also collected; at five minute intervals, the location of each heifer was identified and categorized as within bull pen 1, 2, or 3, or outside of the bull pens, within the enclosure area. A heifer was considered to be within a bull pen if her head and body to the point of her shoulder was within the pen.

To investigate bull-seeking behaviour, two measures were calculated for each heifer:

1) Frequency of entry into the bull pens
2) Proportion of scan samples on which the heifer was located in any of the bull pens.

To investigate female choice, three measures were calculated for each estrous heifer:

1) Frequency of entries into each bull pen
2) Proportion of scan samples on which the heifer was located within each bull pen
3) Frequency of mounting behaviours with each bull including attempted mounts; mounts with the heifer standing; and mounts with intromission and ejaculation.

Because few mounts were observed, the pen entry and scan sample data were used as the primary indices of bull preference.

Statistical Analyses

Experiment 1

From the test female location data collected, the amount of time spent in the various arena sectors (0A, 0B, 1A, 1B etc.) was calculated. The time spent in sectors 1A, 2A, 3A and 4A were used as indicating a preference for being near the stimulus animal(s) and the time spent in the other sectors was used to indicate a preference to be away from the stimulus animals. For each test female the percentage time spent near and away from the stimulus animals was calculated. In an attempt to adjust for the difference in the size of these two zones ("near" was 279.48 m^2 and "away" was 1950.88 m^2) of the arena, the percentage time in the two zones was divided by the area (m^2) of the zone to give 'percentage time per m^2'. A second analysis used the percentage time that the test females spent in each of the arena sectors and for each test female this was then reduced to the percentage time spent near the group-mates and the percentage time spent near the bull with which the test female spent most time (i.e. the most preferred bull). The data were examined using REML in GenStat to assess the effects of the following factors on percentage time:

1. Whether the test female was a heifer or cow;
2. The number of calves the test female had with her (0, 1 or 2);
3. Whether the test female was in natural or stimulated oestrus; and
4. Preference (group-mates or the most preferred bull).

A further analysis examined whether an equal percentage of time was spent by the test females with each bull. We did not necessarily expect all test females to show a preference for the same bull and, so, for each test female the percentage time spent near to a bull was ranked from 1 to 3, with 1 indicating the highest percentage time and 3 the lowest. If the females showed no preference we would have expected the effect of rank to be non-significant. To stabilize the variance, percentage time was subjected to a log (percentage time + 1) transformation prior to analysis. Again REML in GenStat was used to assess the effect of the following factors on percentage time:

1. Whether the test female was a heifer or cow;
2. The number of calves the test female had with her (0, 1 or 2);
3. Whether the test female was in natural or stimulated oestrus; and
4. Rank (1, 2 or 3).

Finally, a one-way ANOVA was conducted to determine if the test females did show an overall preference for one bull. This test was conducted on both the percentage of the total time (1 h) that the females spent with each bull, and with the percentage time spent with each bull as a percentage of the time they spent near bulls. For the latter analysis, one female was excluded since she spent no time near bulls at all.

Experiment 2

All data were analyzed using Statistix, Version 7.0. (Analytical Software, USA). Because our data were not normally distributed, we used appropriate non-parametric tests. To determine whether oestrous heifers demonstrate bull-seeking behaviour, we calculated a total rate of entry (per hour) into the bull pens for each heifer. We also calculated the proportion of scan samples in which each heifer was located within one of the bull pens. The Wilcoxon rank sum test was used to compare the treatments (oestrous and non-oestrous) (Lehner, 1996). The experimental unit used for the statistics is each animal; for each oestrous heifer (N=7), we incorporated only entry and scan data

collected during period(s) when the heifer was classified as oestrous, and for the non-oestrous heifers (N=8) we incorporated only entry and scan data from non-oestrous period(s).

For heifers for which we had entry and scan data collected from both oestrous and non-oestrous observation periods, the data were paired and the Wilcoxon signed-rank test was used to compare between oestrous states for each individual animal (Lehner, 1996); 6 pairs of observations were used.

To determine whether oestrous heifers exhibited bull preference, we incorporated data only from observation period(s) when the heifers were classified as oestrous. For each oestrous heifer (N=7), we calculated entry rate (per hour) into each bull pen and the proportion of scans spent within each bull pen relative to the total scans spent in any of the bull pens. For both entry and scan data, the Kruskal-Wallis one-way analysis of variance was used to test for differences between bulls (Lehner, 1996). One oestrous heifer was not located within a bull pen on any of the scans; therefore, the data from this individual was not incorporated into calculation of the Kruskal-Wallis statistic for the scan data. To determine whether preference for pen location may have had a significant effect on bull preference, the data were organized by pen location, rather than bull identification, and the Kruskal-Wallis one way analysis of variance (ANOVA) was used to test the entry and scan data for differences between pen locations.

Results

Experiment 1

The means and ranges for time near and away the stimulus animals were 56.2% and 0 to 97.9% (SD = 29.6%), and 43.8%, 2.1 to 100% (SD = 29.6%) respectively. When adjusted for the size of the two zones, the mean and range for near and away were 0.2012 and 0 to 0.3504 (SD = 0.1057), and 0.0224 and 0.0011 to 0.0513 (SD = 0.0152) respectively. A paired t-test showed that the test females spent significantly more time near the stimulus animals than away from them ($t = 6.61$ with 19 d.f.; $P<0.001$).

The REML analyses showed that whether the test female was a heifer or a cow, the number of calves with her and whether oestrus was natural or stimulated were not significant and, so, the final analysis was a one-way ANOVA assessing the effect of preference on percentage time. Preference was significant ($P = 0.003$) and showed that test females spent more time with their group-mates than the most preferred bull (means of 35.8% and 13.6% respectively, SE = 4.9). Only 4 test females spent more time with their preferred bull than their group-mates.

The REML analysis to investigate preference showed that the factors of heifer/cow, number of calves and natural/stimulated oestrus were again not significant and the final analysis was a one-way ANOVA assessing the effect of rank on percentage time. Rank was highly significant ($P<0.001$) indicating that the test females did show a preference for a particular bull and the means are presented in Table 1.

Bull Rank	Mean	Back-transformed mean
1	2.28[a]	8.8

2	1.44[b]	3.2
3	0.75[c]	1.1
SE	0.20	

Table 1. Mean percentage times that test females spent with bulls

Values with different superscript letters indicate that they differ significantly

For both percentage of the total test time (P = 0.458) and percentage of the time spent near bulls (P = 0.866), there was no evidence that one bull was preferred on average by the females. The percentages of the total test time spent with bulls were 9.0%, 6.4% and 5.0% for bulls 27, 57 and 70 respectively.

Experiment 2

Although we predicted oestrous heifers would demonstrate bull-seeking behaviour through increased frequency of entry into the bull pens, there was no significant difference in rate of entry into bull pens between oestrous (1.1834 +/- 0.1514) and non-oestrous (0.9677 +/- 0.1702) heifers (p=0.4175). Similarly, there was no significant difference between oestrous (0.1792 +/- 0.0426) and non-oestrous (0.1647 +/- 0.0257) heifers in terms of the proportion of scan samples the heifers were located within one of the bull pens (p=0.4503). The paired comparison using entry rates from heifers for which we had data collected from both oestrous and non-oestrous observation periods also failed to demonstrate significant differences between oestrous (1.1064 +/- 0.1542) and non-oestrous (1.2626 +/- 0.0655) states (p=0.2945). Similarly, the paired comparison using scan proportion did not demonstrate a significant difference between oestrous (0.1478 +/- 0.0339) and non-oestrous (0.2160 +/- 0.0395) states (p=0.1422).

Entry rate and scan proportion data regarding heifer preference between bulls are shown in Table 2.

Measure	**Bull 1**	**Bull 2**	**Bull 3**
Entry rate (per hour)	**0.3299 (+/- 0.1069)**	**0.5543 (+/- 0.1600)**	**0.2992 (+/- 0.0916)**
Scan proportion	**0.3833 (+/- 0.1646)**	**0.5436 (+/- 0.1637)**	**0.0731 (+/- 0.0321)**

Table 2.Values for individual bulls on two measures of female choice, based on data collected from oestrous heifers: 1) Rate of entry into the bull pen 2) Proportion of scans on which each heifer was within the bull pen. Data shown represent means with standard errors; the Kruskal-Wallis one-way analysis of variance was used to test for differences between means.

Although we predicted oestrous heifers would exhibit mate preference in terms of entry rate into each bull pen, there was no significant difference between bulls (Kruskal-Wallis Statistic=1.2425, p=0.5373). Similarly, the proportion of scans on which oestrous heifers were located within each pen did not differ significantly between bulls (Kruskal-Wallis Statistic=3.1804, p=0.2039).

As shown in Table 3, location may have had influenced bull pen selection by heifers, in terms of the duration of time oestrous heifers spent in each pen. When the data were organized by pen location, the Kruskal-Wallis one way analysis of variance did not demonstrate any difference in entry data between pens (Kruskal-Wallis Statistic = 2.2698, p=0.3215), but there was a significant difference in the proportion of scans on which estrous heifers were located within each bull pen (Kruskal-Wallis Statistic = 7.5485, p=0.0230).

Measure	Pen 1	Pen 2	Pen 3
Entry rate (per hour)	**0.3142**	**0.4067**	**0.3474**
Scan proportion*	**0.3036**	**0.1696**	**0.5432**

Table 3. Data for two measures of preference for pen location, based on data collected from estrous heifers: 1) Rate of entry into the pen by each heifer 2) Proportion of scans on which each heifer was within the pen. Data shown represent means with standard errors; the Kruskal-Wallis one-way analysis of variance was used to test for differences between means. *Scan proportion was determined to be significantly different between the pens (Kruskal-Wallis Statistic = 7.5485, p=0.0230).

The number of heifers that accepted mounts was insufficient for statistical analysis, but of the 4 heifers that accepted mounts, 2 accepted mounts from more than one bull.

Discussion

Experiment 1

The test female cattle spent more time with the stimulus animals than away from them. However, by comparison they spent more time near to their group-mates than to any of the bulls with which they spent the greatest amount of time. This suggests a preference to be with their group-mates rather than with any of the bulls, which was contrary to our hypothesis. It is possible that two stimulus animals are a greater attractant than a single animal, regardless of gender. We had considered using a single group-mate as the stimulus, but thought that a socially isolated female may have become distressed and behaved in a way that influenced the behaviour of the test female. The group-mates may have also been attractive to the oestrous female, as it is known that peri-oestrus females form sexually active groups (Rupp et al., 1977; Blockey, 1978, 1980). Although we had selected stimulus group-mates that were not showing standing oestrus, it is possible that they were at a stage of their oestrous cycle where they would have participated in an SAG. A third reason why the test females appeared to show a preference for their group-mates was that they were possibly avoiding the bulls, but preferred to be with other cattle rather than be on their own (although, with their calf in some cases). The anecdotal observations made during recording indicated that the confinement of the bull in a pen and the prevention of access to each other may have been frustrating for both bulls and females. Initially the bulls were very alert, vocalized,

moved around their pens and appeared to watch the test female, but over time they increasingly lay down and appeared to pay little attention to her. It was on the basis of these observations that we hypothesized that the bull and female needed to have access to each other and interact in order for females to remain close to a bull and show a preference, which resulted in the design of Experiment 2.

Having said that we believed access to bulls to be necessary for females to show a preference, the results from Experiment 1 also showed that the test females did not spend equal time with each bull, but appeared to be showing a preference although the amount of time spent near bulls was low. Although we did not necessarily believe that all females would show a preference for the same bull, we deliberately selected bulls of different ages and liveweights to determine whether these factors appeared to influence preference. There is some evidence that oestrous females prefer socially dominant bulls (Blockey, 1979) and social dominance is reported to be correlated with bull age, as a consequence of seniority, or time in the herd (Blockey, 1979) and there is usually a correlation between age and liveweight. Our results showed that there was no overall preference by females for a particular bull, although non-significantly more time was spent with bull 27, which was the oldest and heaviest bull.

Experiment 2

Bull training

Aversive training with audio and electrical stimuli is an accepted technique for training dogs. It has also been used successfully for training cattle, primarily as a means of livestock control. For example, Quigley et al (1990) demonstrated the use of remotely controlled audio and electrical stimulation to train cattle to avoid an area without a fence-defined boundary. This experiment demonstrates the first documented application of training with audio and electrical stimuli as a means of confinement for beef cattle, specifically bulls. It appears to be an effective training technique to confine bulls without physical restraint. During the initial training session, the bulls each received only 1 or 2 electrical stimuli before they appeared to respect the boundary represented by the painted lines on the ground. The bulls also appeared to respond effectively to the audio stimuli, without the need for the electric stimuli; by the third day of training, all of the bulls were observed to turn or stop at the painted lines upon receiving an audio signal. Once the female choice experiment began, the bulls were more highly motivated to exit the bull pens in pursuit of oestrous heifers. However, the training remained effective. During the observation periods, bulls were observed to stand in close proximity to, but not cross, the painted lines, and the use of audio or electronic stimulation was only required on a few occasions to maintain the bulls in their pens.

Bull-Seeking Behaviour

Descriptive studies have implied that oestrous cows demonstrate bull-seeking behaviour (see Blockey, 1979; Fraser, 1985; Houpt, 1987), as they approach receptivity, cows are thought to gradually become more attentive to bulls, seek positional proximity to bulls and to actively participate in courtship interactions. However, our results did not

demonstrate a significant difference between estrous and non-estrous cows in terms of the frequency or duration of visits to bulls.

Oestrous females may not exhibit bull-seeking behaviour. It is possible that the active female role in initiating courtship consists of advertising receptivity through behavioural displays and the formation of SAGs. In his investigation of estrous detection by bulls, Blockey (1978, 1980) reported that in 17 of 22 observations, the bull approached and joined the SAG immediately after female-female mounting occurred within the group, whereas in only 4 instances the group approached the bull. Similarly, in the current study, oestrous heifers consistently displayed receptivity in the sense that they stood to be mounted, but did so largely outside of the bull pens (by the SAG), which suggests that they may have been expecting the bulls to approach and initiate courtship.

Alternatively, the heifers may have lacked the sexual maturity or experience necessary for the expression of bull-seeking behaviour, but more likely, the study design may have interfered with the expression of bull-seeking behaviour. For example, estrous females may exhibit bull-seeking activity only in response to specific physiological or external stimuli. Ram-seeking behaviour in ewes depends on external stimuli provided by rams (i.e. sight and sound of ram), as well as an internal stimulus of estrogen secretion (Fletcher and Lindsay, 1968; Lindsay and Fletcher, 1972). Confinement of the bulls may have interfered with stimuli from the bulls necessary to stimulate bull-seeking behaviour amongst estrous females. The behaviour of the bulls in response to confinement may also have deterred the heifers from entering or remaining within the bullpens. The bulls, particularly the oldest, most dominant bull, paced and vocalized at the front of their pens throughout the observation periods; if the heifers interpreted such behaviour as abnormal or threatening, they may have avoided the bullpens as a result.

Female choice

Numerous studies from many taxa have concluded that males possessing certain attributes have a mating advantage arising from female mate choice, particularly amongst polygynous species in which parental investment by males is minimal (Jennions and Petrie, 1997). In cattle, the only parental investment provided by the bull is to inseminate and guard the cow during oestrus and prevent her from being mated by rival bulls (Phillips, 2002). Therefore, it is reasonable to expect that cows should exhibit mate choice in order to maximize their reproductive success. However, studying contrast to Experiment 1, this experiment found no significant difference between bulls, in terms of the duration or frequency of visits by oestrous heifers. However, although not statistically significant, the entry and scan data both indicated that oestrous heifers preferred the intermediate bull (Bull 2), which contrasted the predicted outcome, in that the intermediate bull was neither the oldest or largest of the bulls.

Female choice may not be a significant determinant of mating success in cattle. Costs, including increased energy and time expenditure and increased predation risk, are often associated with female mate choice in many species. Consequently, females of some species maximize their reproductive success by mating with multiple males.

Females adopt this strategy in order to generate sperm competition, particularly in species where the males lack phenotypic traits that are reliable indicators of genetic quality, or as mechanism to reduce inbreeding (review: Jennions and Petrie, 1997). There were insufficient services by bulls during this study to permit analysis of the prevalence of multiple mating. However, numerous studies have reported that when multiple males are used in breeding groups, 50% or more of females are serviced by 2 or more bulls (Mattner et al., 1974; Rupp et al., 1977; Farin et al., 1982), which suggests that accepting services from multiple bulls may be a significant reproductive tactic in cattle.

The lack of female choice exhibited by the heifers in this study may also be a reflection of the operational sex ratio (OSR) of the two sexes. The OSR can influence sampling tactics and mate-choice decisions; for instance, when the density of the chosen sex is lower, there may be increased costs associated with sampling, or increased risk of failure to mate, leading to a reduction in choosiness by the choosy sex (Jennions and Petrie, 1997). If we are to assume that cows do, in fact, exhibit choice, it bears consideration that the OSR of the current study may have favored a reduction in female choosiness. Furthermore, theoretical discussions have suggested that the OSR is significant in the evolution of mating systems; therefore, female choice may not have evolved in cattle due to a skewed OSR under natural conditions, or traditional breeding practices, wherein a large number of cows are maintained with only one or a few bulls, may have selected for a reduction of female choosiness.

It is possible that our experimental design (in both experiments) interfered with the basis on which females make their choice. For example, females may incite or induce competition between males and require an outcome to such competitions to ensure mating with the most dominant male. A female may provoke intra-male competition by providing physiological and/or behavioural signals that alert males in the area, either causing them to compete amongst themselves for access to her, or causing them to prevent copulation by other, subordinate, males (Cox and Le Boeuf, 1977). Cows approaching estrous demonstrate increased frequency of urination and bulls respond by sampling the urine (Phillips, 2002); however, it is not known whether urine acts simply to advertise receptivity, or whether it may also act to stimulate intra-male competition. In a herd, as more individuals approach estrus, the frequency of cow-cow mounting increases, and one or more sexual active group(s) (SAG) form (Blockey, 1978, 1980). Such behaviours, which imitate a rival male copulating with the estrous female, serve to attract males in many species in which intense intra-male competition occurs (Parker and Pearson, 1976); they may also act as a mate choice tactic to incite intra-male competition. Confining the bulls would have eliminated this strategy, which is thought to be an important component of reproductive behaviour in some ungulates (Cox and Le Boeuf, 1977).

Female choice may also operate through avoidance behaviour. The current study, and most research concerning female preference, have considered positive responses by the female (ie. moving towards a male) to be an indication of choice; however, preference may also be demonstrated through avoidance or rejection of undesirable mates. For example, a female's resistance to copulatory mounts may be due not only to non-receptivity, as is well-recognized in cattle, but also contingent on the identity of the suitor

(Cox and LeBoeuf, 1977). For instance, Poole (1989) reported that female elephants exercised choice by actively avoiding copulation by younger males. Stookey (J.M. Stookey, pers. comm.) has documented via a series of photographs an example where a cow was observed to determinately avoid being sequestered by a low-ranking bull, which suggests that cows may use also avoidance behaviour as a mate choice tactic.

It is interesting that when the data was organized by pen location rather than bull identification, there was a significant difference in the proportion of scans spent within, but not entry rate into, the bull pens. This might suggest that females exhibit a preference for territory, but bulls are not thought to defend territories; a bull will actively 'guard' an estrous cow during courtship but he does not drive her to a specific mating site (Albright and Arave, 1997). However, a recent study by Fordyce et al. (2002) suggested that individual bulls have home ranges, in which they spend a large proportion of their time, but which can also change in size depending upon the location of females and the number of bulls in the area. It seems more likely that the heifers in this experiment were selecting for an environmental feature of the preferred pen. Maximum daily temperatures were in the range of 25 to 30° C during the observation periods (Environment Canada, 2005), and only the preferred pen (pen 3) contained a shaded area, in which a number of the heifers were observed to rest, so it seems likely that this is the explanation for the difference in scan sample results.

The results from Experiment 2 were similar to those from Experiment 1, in that there was no evidence that oestrous females sought and spent more time with bulls than non-oestrous females, although it is difficult to draw direct comparisons because in this experiment the test females were not offered the choice of group-mates.

Conclusions

These experiments demonstrated that oestrous females appeared not to exhibit bull-seeking behaviour, but may have a preference to remain with group-mates. The first experiment indicated that oestrous females did not spend the same amount of time with all bulls, but showed some preference, although on average no one bull was favoured. However, experiment 2 did not reveal preferences for bulls, but rather for a particular pen. It may be that female cattle select mates on the basis of the outcome of bull social interactions or on the quality of their territories or home ranges. Alternatively, it may be that the practice of having low bull to female ratios in beef breeding herds has resulted in females being less discerning in their choice of mate and/or mating with multiple bulls allows sperm competition to occur.

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A study on the behaviour of bulls on community pasture using GPS collars. Amanda Brown, Joseph M. Stookey, Jon Watts and Doug Veira

Background:

Previously our observations on the behaviour of bulls and cows on community pasture led us to hypothesize that some cows may avoid breeding or preferentially

breeding with specific bulls. As a result we tested this hypothesis in controlled studies where we limited the movement of bulls, but allowed females to "choose" a preferred bull without interference from all bulls within the group (see previous study on mate choice). Another way to explain the differences we see in mating success of bulls at pasture is to look for behavioural differences in the amount of travel or areas occupied by bulls in the community pasture, social status or age differences among bulls. Several avenues have been investigated by our group and this study looked at the data collected from GPS collars worn by bulls in a community pasture during the breeding season.

Methodology:

Data was collected at the Rudy-Rosedale community pasture. During the period from 4th June to 5th July 2004, 11 bulls wore GPS collars intended to log their position every 10 minutes. Ideally this would have given us approximately 4600 position plots per animal. In practice this is unlikely to be realized for any individual, and such was the case in this study. A variety of factors contribute to a loss of useable data. Some data were lost during post-processing and differential correction due to excessive dilution of precision (DOP). In other words there is an unacceptable degree of uncertainty about the accuracy of a fix. This happens because of eccentricities in the actual GPS satellite system. Another source of loss occurs when the collar fails to detect signals from the satellites in orbit. The collar attempts repeatedly to obtain a fix, but ultimately a timeout can occur, meaning that the scheduled position fix is never obtained. Data can be lost due to the animal being in dense bush, or in a particular posture that blocks the collar's "view" of satellites. The dropout may be temporary and relatively insignificant. However, if it happens chronically because of poor placement of the receiver, low battery power or a malfunctioning antenna, a serious loss of data can occur. We experienced both types of losses in this study (the inability of the satellite/collar to take a fix and collar malfunction). Other data were deleted for irrelevance, for example, when the animal was being transported to, from and between study areas. In a number of cases a GPS collars became detached from an animal for up to several days before it could be replaced. Overall, the performance of the GPS units and retention of the collars was disappointing considering 11 bulls were fitted with collars. Consequently, the amount of useable data obtained was also disappointing. The most complete set of data originated from only 4 bulls during the first 15 days of the study, from whom we obtained reasonably complete data sets.

Distance traveled

The distance separating each 10 min interval GPS fix from the previous one for each bull was determined using a Pythagorean geometrical approximation. This gives us a minimum distance in meters moved by the bull between fixes. A summary of daily distances traveled for one bull (Bull 02) for whom a nearly complete record is available is shown in Table 1. Remember that because of inaccuracies in the GPS system, and the way the collar and satellite interact to fix a data point, we can obtain differences in the reported position of up to 10m between fixes even if the bull did not move at all. Over many fixes though, this method should yield a reasonably accurate lower estimate of bull movement during the period. The values obtained are within the range of accepted estimates of bull daily travel.

DAY	Daily total distance traveled (m)	Daily mean distance between GPS fixes (m)	Std. Error of Mean (m)	Minimum (m)	Maximum (m)
1	15283.02	107.6269	13.55805	.47	752.34
2	7834.89	54.4090	7.57223	.26	492.92
3	8592.57	60.0879	7.88413	.26	476.94
4	5034.33	35.4530	6.06150	.36	380.92
5	10673.50	75.1655	10.96466	.43	798.98
6	6620.49	46.6232	6.27506	1.11	422.87
7	11401.91	80.2952	11.63023	.43	686.20
8	10801.97	76.6097	10.08654	.26	590.74
9	9431.04	65.9513	8.00606	.22	570.21
10	11803.09	87.4303	10.61964	.75	750.30
11	7137.07	50.6175	7.78764	.59	575.58
12	10231.40	79.3131	8.33163	.47	443.54
13	13525.93	94.5869	10.55097	.33	579.05
14	5948.12	41.5953	7.06157	1.80	700.68
15	8561.05	62.9489	7.21175	.23	507.70
16	13899.67	99.2833	10.34718	.43	734.11
18	11534.02	80.6575	10.13739	.18	654.46
19	7311.44	51.8542	7.63687	.28	579.08
20	5412.90	45.4866	6.66683	.24	462.28
21	5977.52	41.5106	6.08244	.36	507.86
22	6303.40	45.6768	8.41688	.26	588.72
23	4796.37	34.7563	5.01989	.34	382.43
24	2952.18	21.2387	4.82120	.18	454.13
25	2768.55	19.4968	3.55178	.23	257.40
26	6125.63	44.3886	7.75784	.26	675.62
27	8697.41	61.2494	8.26729	.18	559.51
28	7816.23	55.0439	7.84031	.13	436.34
29	9284.17	64.9243	10.56685	.28	718.51
Grand total	235759.87	60.1889	1.65128	.13	798.98

Table 1. Record of daily distance moved by Bull # 02. Note that Day 17 data are excluded because the bulls were moved between pastures on that day.

Bull positions within the 2 section pasture were plotted using ArcGIS software as illustrated in Figure 1. The distance between position plots was calculated using SPSS statistical software.

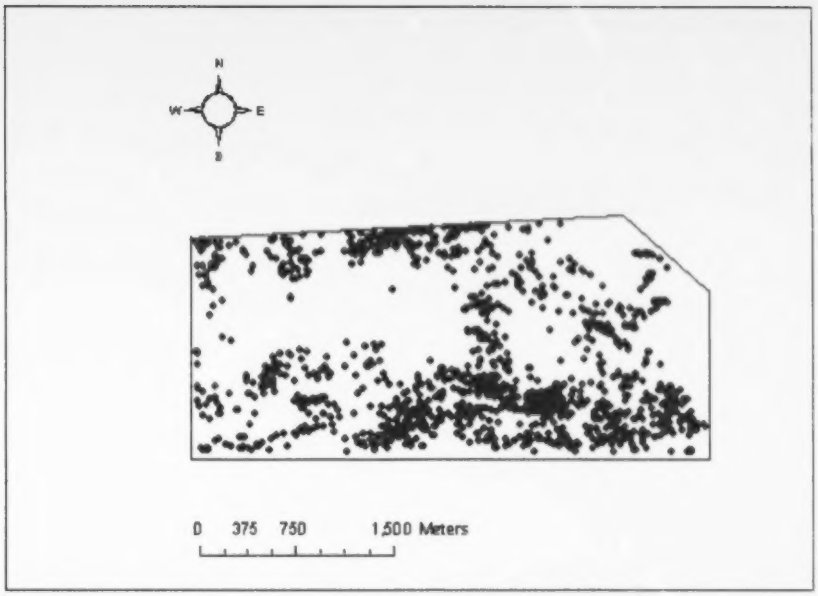

Figure 1. GPS position plots for one individual bull (Bull 02) within the field perimeter.

Mean minimum daily distance estimates are given in Table 2. Statistically there was no difference between bulls in the total distance traveled. It is also obvious that the bulls apparently utilized or occupied the bulk of the area available to them and there is no obvious evidence that certain bulls were occupying a "home range" or fixed territory in an area of about 8 quarter sections. Bulls may have had a preferred area, but nonetheless they logged data points throughout the available area. Theoretically they could have bred cows in any area of the pasture based on their use of the area. It means that differences in breeding success of the bulls is more likely a function of age, social status or behaviour, then it is to limits in their range over the geographic area.

Further analysis

Although the GPS data are incomplete, there are a number of tentative hypotheses that we considered worth exploring. In particular were interested in the relationship between the age and dominance ranking of bulls and their spatial behaviour. While it may be the case that bulls travel similar distances, it may be that more locomotions (and perhaps also breeding activity) occur at night for some individuals. We also planned to examine the amount of space occupied by each bull within a given time interval to determine whether there is evidence to support the idea that some individuals maintain a core home range or territory within which they attempt to control breeding access to females. This is not apparent from the data set. Our tentative hypothesis is that dominant males may operate within preferred areas while subordinates move opportunistically throughout the available pasture area.

BULL ID	Age	Mean daily distance (m)	Std. Error of Mean (m)	Minimum (m)	Maximum (m)
# 212	2 years	10071.800	420.44829	7592.00	12225.00
# 48	3 years	9378.6000	847.67272	1123.00	15658.00
# 02	4 years	9524.9333	733.81398	5034.00	15283.00
# 05	4 years	10112.375	726.43299	7150.00	13289.00
Total	9726.9623	9726.9623	349.19352	1123.00	15658.00

Table 2. Mean distance traveled for four bulls measured over 15 days.

While the GPS data was somewhat discouraging in that not all the collars yielded usable data, a consoling aspect of the study was that we were able to access the collars from Canada Ag and Food (Doug Vierra) and as a result did not have to purchase or lease the collars. Each collar costs over $5,000 and through the collaboration of Canada Ag and Food researchers we were able use equipment worth in excess of $50,000, plus tapped into their expertise in downloading and working with the data. It was a good deal for us and Saskatchewan ADF in being able to conduct "expensive research" without equipment costs!

Two-stage Weaning:

Introduction: Previous work in our lab has shown that two-stage weaning nearly eliminates the behavioural signs of distress (calling, walking, reduced eating, etc.) that are typically seen following abrupt weaning. The two-stage weaning in some ways mimics natural weaning in that the cow and calf remain together during stage 1 when milk is no longer available to the calf. The milk deprivation is accomplished with the use of an anti-sucking device which is attached to the calf's nostrils and acts as a physical barrier preventing the calf from sucking on the teats. After 4-7 days of milk restriction the cows and calves can be successfully separated with minimum signs of distress. The procedure is gaining acceptance within the cattle industry with an estimated 70,000 calves weaned in 2004 using this technique (estimates based on Quietwean nose tag sales in 2003-2004).

Two studies were initiated in the fall of 2004 to take advantage of the two stage weaning procedure.

Trial 1. In collaboration with our lab, researchers from VIDO are searching for blood borne proteins associated with weaning stress. The relatively new area of proteomics will allow us to identify factors within the blood serum that are unique to weaning stress.

Aim of Study:

1. Determine if the overt behavioral changes associated with the psychological stress of abrupt weaning are associated with significant physiological changes.

2. Determine if the elimination of behavioral changes during two-stage weaning is associated with a significant reduction in physiological changes.

3. If significant physiological changes can be measured during weaning then these changes will be analyzed to determine how they may relate to known mediators of stress responses, such as cortisol.

4. Identify and characterize biomarkers of stress

Experimental Design:

The study used 40 calf/cow pairs Calves vaccinated (8-way Clostridial; MLV BVD/PI3/BHV-1) two weeks prior to initiating the study to minimize potential affects of respiratory viral infections during the weaning period. All cow/calf pairs are housed in a single pen throughout the study and separation of cow and calf (weaning) will be performed by removing selected cows from the pen. Thus, the stress of weaning should be specific to disruption of the maternal/nutritional bond with no associated effect due to transport, environmental change, or social mixing. Assignment of calves to groups was done by formal randomization (random number table). Laboratory group will be blind to the experimental groups until sample analysis is complete.

Four experimental groups (n = 10) have been defined:

Group I - Never Weaned (NW): Calves remain with their mothers throughout the study. These calves provide controls for possible effects due to handling during sample collection and provide a comparison with suckling calves.

Group II - Pre-conditioned (PC): Cows removed from calves (traditionally weaned) two weeks prior to the start of the trial. This two week period has been selected as an appropriate interval to eliminate potential psychological and physiological effects associated with breaking the maternal/nutritional bond.

Group III - Abrupt Weaned (AW): Cows will be removed from the calves at the beginning of the experimental sample collection (Experimental day 14). This group should display the full range of behavioral responses associated with breaking the maternal/nutritional bond and the most pronounced physiological changes if these occur during weaning.

Group IV - Two-Stage Weaned (2S): Calves will be fitted with plastic nose paddles (Experimental Day 10) for four days prior to removal of their mothers from the pen (Experimental day 14). This group should provide samples to quantify physiological changes associated with dietary changes (Experimental Days 10-14) and samples to determine if a significant reduction in overt behavioral changes associated with maternal separation also results in a significant reduction in physiological changes associated with breaking the maternal/nutritional bond during abrupt weaning.

Methodology for measuring physiological changes associated with the stress of weaning.

1) Proteomic analysis of changes in serum proteins. We hypothesize that changes in serum proteins will reflect physiological changes in many tissues (i.e liver, muscle, etc.) that respond to changes on glucocorticoid levels. This hypothesis is also based on the assumption that the psychological stress of weaning will induce a significant increase in glucocorticoids and other mediators of stress responses.

2) Microarray analysis of changes in gene expression in blood leukocytes. We hypothesize that during the stress response associated with abrupt weaning there will be significant changes in blood leukocyte subpopulations and changes in the function of these cells which will be reflected in significant changes in gene expression.

Progress to date: The treatments were imposed and blood samples were collected form the groups in November, 2004. Blood analysis and the search for specific stress markers are still underway at the time of this report and no data is available. The search for protein markers is a complex and time consuming procedure. Only 12 gels can be run per week in the lab. The protein spikes and magnitude of the spikes on the gel must then be compared to the values obtained from other samples to determine if treatment differences occur. We are still optimistic that new markers (proteins) can be found that represent a biological response to stressors. Once the new "stress proteins" are identified we will know whether calves weaned following the 2-stage weaning protocol or experiences similar or reduced stress compared to the traditional abrupt weaning procedure.

Using the Two-stage weaning anti-sucking device as a vehicle to deliver vaccines.
Joseph M. Stookey, Albert Barth and Derek Haley.

Recently, we developed a two-stage method for weaning beef calves that essentially eliminates the behavioural signs of weaning distress that normally accompany the traditional method of weaning by abrupt separation (Haley et al., 2005. J. Anim. Sci 83, 2205-2214). We believe by eliminating weaning distress calves may have a health advantage when leaving the farm. Improved health for newly weaned calves would be a benefit since bovine respiratory disease or shipping fever is the single most important health problem in Canadian feedlots (Jim et al., 1999: Can Vet J 40:179-184) and is essentially a disease of newly arrived and newly weaned cattle. Roughly 30% of feedlot calves are treated for respiratory diseases. It is reported that up to 80% of the morbidity and 55% of the mortality within the feedlot occurs within 45 days of arrival with respiratory disease accounting for over 80% of the losses (Smith, 1998: JAS 76:272-274).

What factors contribute to the high incidence of respiratory disease in newly arrived feedlot calves? One contributing factor is likely the fact that the bulk of calves are weaned and sent directly to market on the same day as weaning. It is estimated that over 40% of the operations send calves directly off the cows to market (see Figure 1; USDA, 1997). One distinct advantage of the Two-stage weaning procedure is that even

if producers send calves to market directly off of cows, the calves will have been already weaned while in the presence of the cows, thereby eliminating the weaning stress that the bulk of calves experience during the first week at the feedlot.

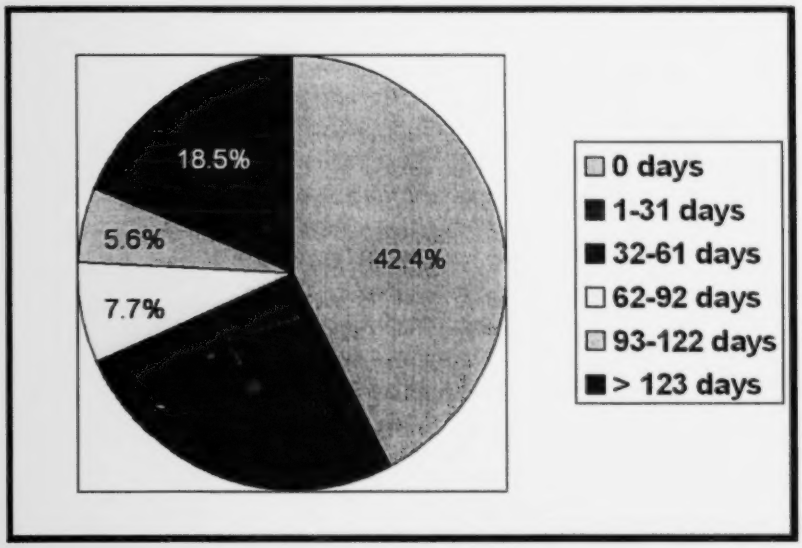

Figure 1. Percent of operations by number of days weaned calves were held before sold *(USDA 1997)*.

Another factor contributing to the high incidence of shipping fever is that the majority of calves sold directly off farms and ranch have never been vaccinated for respiratory diseases prior to sale (see Figure 2; USDA, 1997). Probably because of this lack of protection feedlots routinely vaccinate calves upon arrival, however for vaccinations to be effective against diseases they require a recommended 14 days to be on-board before the animal should come in contact with the disease. We also know that for calves to launch a good immune response to vaccines that they should be stress free. However, newly weaned and newly arrived calves at a feedlot are certainly not stress free. In addition, the best possible protection for vaccines is obtained by vaccinating the animal twice (once for the primary response and later with a booster shot). Based on data from USDA (see Figure 2) an estimated 86% of operations are selling calves that are inadequately protected for respiratory diseases when they reach the feedlot.

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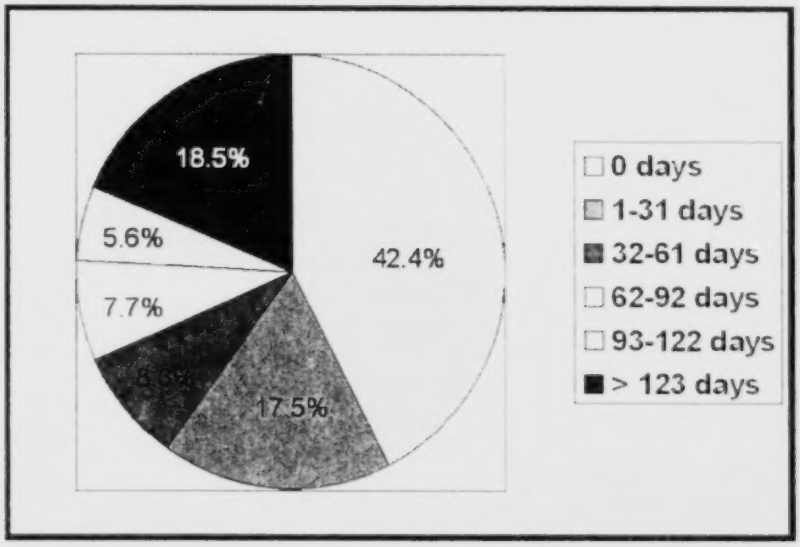

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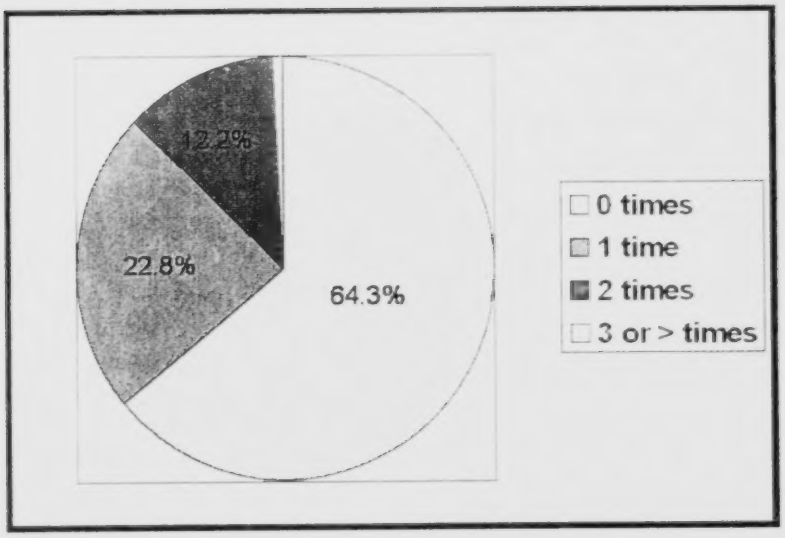
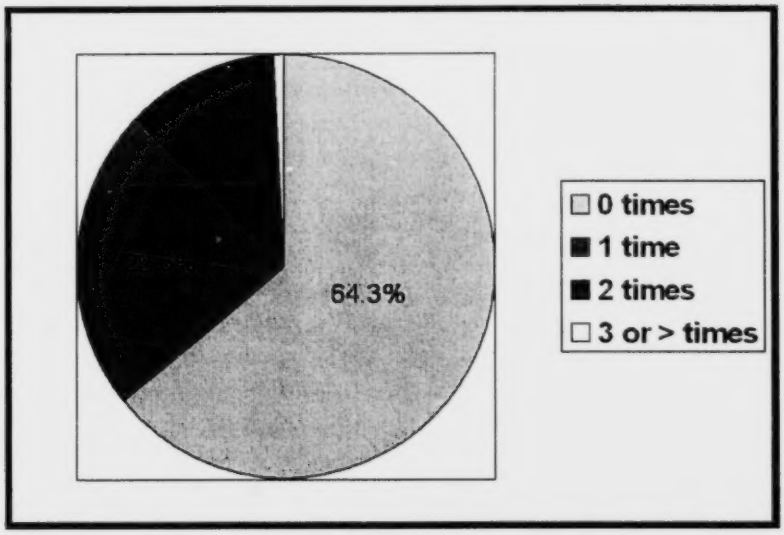


Figure 2. Percent of operations by number of times calves were vaccinated for respiratory disease before sale *(USDA 1997)*.

We know that a significant number of producers in Canada and USA are adopting two-stage weaning. Based on sales of the Quietwean anti sucking device, conservative estimates of over 100,000 calves were weaned using these nose tags in 2005. Estimates of calves weaned using the Servet anti-sucking tag are unavailable, but likely exceed Quietwean sales. One way to help protect calves from the stress of weaning and protect them from respiratory disease would be to vaccinate calves at the same time as producers handle calves during the Two-stage weaning procedure. The two-stage weaning procedure requires that calves be caught and restrained twice during the weaning procedure; once when the anti-sucking devices are inserted and a second time when the anti-sucking devices are removed. However, most producers will normally handle their calves at least once during weaning anyway, either to vaccinate, tag, weigh, etc. or to separate from the cows. Already many producers using the two-stage weaning technique are coupling the insertion of the anti-sucking device with their vaccination protocol. We wondered whether the anti-sucking device itself (Quietwean) could act as a carrier to deliver the vaccine. This would have the advantage of avoiding another injection, plus could take advantage of the intranasal vaccine technology already developed. Plus the Quietwean nose tag, if used to deliver a vaccine, may have an advantage over other intranasal vaccines in that the vaccine could be "held in place" with the anti-sucking device against the mucosal lining.

Methodology: A total of 40 Quietwean nose tags were modified by drilling a cavity into both nose tongs to hold either IBR or BVD vaccines suspended in a gel. One hundred calves were used in the trail and all calves had blood samples taken at the start of the trial for use in establishing baseline titer values and again 14 days after vaccination. All calves were weaned following the two-stage weaning technique using Quietwean anti-sucking nose tags.



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Calves were randomly assigned to one of the following five treatments.
1) 20 calves vaccinated with IBR imbedded into Quietwean nose tags + 8-way subcutaneous injection.
2) 20 calves vaccinated with BVD imbedded into Quietwean nose tags + 8-way subcutaneous injection.
3) 20 calves vaccinated with IBR intranasal + 8-way and control Quietwean nose tags
4) 20 calves vaccinated with BVD + 8-way subcutaneous injections and control Quietwean nose tags.
5) 20 calves vaccinated with only 8-way subcutaneous injections with Quietwean nose tags.

Calves were blood sampled, vaccinated and Quietwean nose tags were inserted on Oct. 21st. Quietwean tags were removed and calves were separated from cows on Oct 25th. Calves were blood sampled again on Nov. 5th.

Results: Titers for the vaccines were submitted form a sub sample (n=10/trt) of the entire group and result appear below.

Pfizer TSV-2: IBR, PI3 intranasal vaccine
Pfizer Bovi-Shield 3: IBR, BVD, PI3 intramuscular vaccine

TSV-2 tag			**TSV-2 nasal**			**BoviShd tag**			**BoviShd IM**			**Control**		
ID	21-Oct	5-Nov	ID	21-Oct	5-Nov	ID	21-Oct	5-Nov	ID	21-Oct	5-Nov	ID	21-Oct	N
22	2	6	**9**	1	4	**35**	1	40	**34**	3	24	**20**	3	
44	1	10	**25**	7	4	**85**	1	42	**41**	1	26	**67**	2	
53	5	5	**26**	2	2	**108**	2	25	**83**	14	11	**76**	40	4
55	1	3	**27**	3	2	**127**	4	27	**105**	3	26	**102**	3	
63	10	9	**66**	8	5	**129**	3	17	**109**	6	8	**145**	5	
65	3	5	**81**	3	2	**165**	2	52	**124**	3	8	**147**	6	
101	4	10	**91**	2	13	**166**	1	44	**151**	1	32	**150**	1	
111	6	4	**113**	1	2	**182**	2	44	**176**	0	41	**152**	1	
118	4	5	**138**	3	2	**184**	1	40	**180**	1	23	**158**	5	
121	10	7	**154**	0	1	**192**	4	25	**181**	1	14	**168**	1	

Each vaccine was tested using two methods of delivery: either mixed in a gel and inserted into a cavity in the Quietwean tag or delivered following the recommended technique developed by the vaccine company, either intranasal (for TSV-2) or intramuscular injection (for Bovishield). The results are very promising in that in both cases the vaccine delivered via the Quietwean nose tags was as good as or better in launching a response than the same vaccine when administered in the recommended way. It means that the Quietwean nose tag in addition to weaning the calves could serve as a vehicle for delivering vaccines in a needless manner. If commercially developed it means that weaning and vaccinations could all be done in one simple step of inserting the nose tags.

Handler behaviour can influence the amount of struggling by beef cattle during restraint. Jane Noble, Joseph M. Stookey, Jon M. Watts, Danica Olenick, and Richelle Kendall

Abstract

Animals make associations between their actions and the consequences. They can also mistakenly associate an outcome with their behaviour, when the actual events are causally unrelated; sometimes called "superstitious learning". Typically, cattle are released from a headgate or crush when the procedure for which they were restrained is finished, however, they may attribute their release to the behaviour they performed immediately before. Thus, an animal that was struggling when released may learn to struggle more during subsequent restraint. We investigated whether we could exploit the associative learning ability of cattle to alter their subsequent response to restraint. Forty-eight beef heifers (634.1 ± 7.3 kg), naïve to the handling system, were individually scored for temperament (1-3 scale) during isolation on a weigh scale. They were then randomly allotted, based on temperament, to two treatment groups in which they had to meet specific behavioural criteria prior to release from a headgate. In one treatment (Struggle), animals were released when they were pulling or pushing against the headgate. In the other treatment (Calm), cattle were released when they had stood calmly for 1-2 s. For 5 consecutive days, the heifers were caught and restrained in the headgate for 15 s, after which the animal's assigned treatment and subsequent behaviour determined when it would be released. The animals' responses were recorded on video and exertion force against the headgate was quantified using strain gauges. The time to reach criteria for release significantly decreased between day 1 and day 4 for the Struggle treatment group (D1=12.9±5.3 s, D4=3.8±1.5 s, p=0.01), but not for the Calm group (D1=26.3±5.2 s, D4=21.8±5.9 s, p=0.33). The Struggle group was able to make the association between its behaviour and release, while the Calm group may not have made the association. On day 5 both groups were restrained for and additional 30 s to see if they would maintain their respective release criteria for an extended period of restraint. Both the Struggle and Calm treatment groups exerted less force on the headgate on day 5 compared to day 1 ($D1_{Struggle}$=63.3±8.0 με, $D5_{Struggle}$=43.0±4.6 με, p=0.02; $D1_{Calm}$=40.4±3.4 με, $D4_{Calm}$=31.4±2.5 με, p=0.02). The behaviour of restrained cattle can be influenced by appropriately timing their release from the headgate, but cattle may learn to struggle to obtain release more readily than they learn to remain calm. By releasing cattle only when they are calm, handlers can at least avoid inadvertently training the animals to struggle more during restraint.

1. Introduction

Animals have the capacity to learn, and they can make associations between their actions and subsequent outcomes very quickly. The rapid association between animals' actions and their consequences has been demonstrated in some of the classical learning studies by B.F. Skinner and Edward Thorndike (Pearce, 1997). Thorndike showed that when cats were placed in a box with food outside the door, they would learn that their actions (i.e. depressing a lever) led to their release (Pearce, 1997). Animals also sometimes mistakenly associate their actions with outcomes that are actually caused by events that are beyond their control. Skinner's work with pigeons on a timed feeding schedule,

showed that even though the arrival of food was beyond the birds' control, they would continue to display behaviours (such as pecking at the feed hopper), if these behaviours had been coincident with food release in the past (Roper, 1983). This faulty association is referred to as *superstitious* learning, and there is anecdotal evidence that it can occur in cattle. The increase in vocalization prior to feed delivery may be an example of this phenomenon.

As part of their routine management, cattle are caught in a headgate, subjected to various manipulations, such as vaccination, branding, or pregnancy checking, and released when the task has been completed. These procedures are often considered aversive (Schwartzkopf-Genswein et al., 1997). Moreover, restraint in the headgate is, in itself, probably distressing, and many animals struggle against it. Because of superstitious learning, cattle might associate their release with their own struggling behaviour, rather than with the completion of the procedure for which they were restrained. Thus, an animal released while struggling on one occasion, might struggle harder the next time it is restrained, to try to elicit release.

Hagen and Broom (2004), found that cattle show a positive emotional reaction to having some control over aversive stimuli. Furthermore, studies on rats have shown, that animals with control over aversive stimuli, had decreased instances of stomach ulcers, and experienced better general health than those that did not (reviewed by Drugan *et al* 1997). If cattle are given some degree of control over an adverse situation (such as being restrained in a headgate), this could help to reduce stress and improve health and well being. If it is possible to train cattle to display a desired behaviour during restraint, it would allow us to "give" some control back to the animals we are working with, improving their welfare at the same time as improving ease of handling.
With current production practices, stress from handling is often high during the events preceding slaughter (Le Neindre et al 1996). An animal's reaction to this handling can, in part, determine the degree of bruising and dark cutting on the carcass (Le Neinder et al 1996). Voisinet *et al* (1997a, 1997b) linked wild temperament with decreased average daily gain, and increased instances of borderline dark cutting, and tough beef.

Grandin (1994) reports an increase in the number of cattle that "become highly agitated and excited when restrained or handled" leading to lowered productivity. This can be partly avoided by selectively breeding animals with calm temperaments. There may also be an opportunity to exploit an animal's associative or superstitious learning ability and emotional reaction to its own learning. If an animal can be trained to stand calmly in the headgate in order to gain release, handlers could use less restraint, resulting in decreased risk of injury to both animal and handler and, possibly, increased productivity (Grandin 1999). Antelope and bison have been successfully trained using food rewards to enter a chute or box for blood sampling (Grandin, 2000), but we are unaware of attempts to train cattle to remain calm during restraint by rewarding them with release.

In the present study we tested the hypothesis that the amount of struggle performed by cattle during restraint can be altered by a training regime in which release is contingent upon meeting a criterion of either calm or struggling behaviour.

2. Methods and Materials

The handling and restraint procedures described in this article were approved by the University of Saskatchewan's Animal Care Protocol Review Committee (UCACS #20050043).

2.1 Animals and Pre-test Temperament Scoring

A group of 48 mature crossbred beef heifers (634.1±7.3 kg) of unknown breed origins were purchased from a local auction market and used in the study. Within 2 days of arrival at the University of Saskatchewan's Goodale Research Farm, they were subjected to a pre- test temperament evaluation, before being allocated to treatments, to ensure that tne two treatment groups were evenly balanced with animals of similar dispositions. All the heifers were naïve to the layout and handling systems. Heifers were moved from an outside holding pen, through a chute complex that led them indoors where they were individually isolated for 1 minute on the weigh scale. While on the scale platform they were video recorded using overhead cameras. Their individual reactions to isolation were later played back and scored on a 3-point scale (modified from the 5 point scale used by Voisinet et al 1997). A score of 1 was given to animals that stood calmly, with little or no movement. Animals scored 2 if they showed some movement on the scale, but the movements were not violent or jarring. A score of 3 was given to those animals that exhibited movement that was constant, violent, jarring, or frenzied. After assigning the temperament scores, animals were randomly allocated to treatments so that equal numbers of heifers with each temperament score were present in each treatment group

2.2 Treatment groups

There were two treatments, with 24 animals assigned to each. Both treatment groups were subjected to handling, as described below (see section 2.4), which included capture in, and release from, a headgate. Handling of the two treatment groups differed only in the behavioural criteria required from the animals before they were released. Animals in one treatment group (designated the "Calm" treatment), were released from the headgate after they appeared to no longer push or pull against the headgate for 1- 2 consecutive seconds, as judged by the headgate operator. Animals in the other treatment group (designated the "Struggle" treatment) were released when they were observed to be actively pushing or pulling against the headgate for 1-2 consecutive seconds, as judged by the headgate operator.

2.3 Handling equipment and strain gauge system

The handling system consisted of a curved metal chute outside, leading up to the door of the testing shed, where it connected to a straight chute inside, equipped with a single animal, weigh scale (Sensitek, model: Sensi-weigh), and a manual, self-catching headgate (Pearson Livestock Equipment, model: GS, USA). To quantify the exertion forces, two patches, each consisting of two strain gauges (Micro-Measurements, model: CEA-06-062UT-350, USA), were fixed at an animal's neck height to the headgate using an epoxy adhesive according to the manufacturers instructions to form a "full bridge" circuit. The strain gauges were connected to a data logger (Campbell Scientific, Inc., model: 21X, USA) and laptop computer (Toshiba, model: PT831C-67CU2, XP Professional operating system, China). The strain gauges were

designed to withstand exertion forces of ± 1500 microstrain (με). Campbell Scientific PC 208W software was used for communication between the data logger and the computer. The strain gauges were covered in foam padding which was secured in place using duct tape. Video cameras were used to record the animals within the scale section and at the headgate. Red and green lights (Lamps: Catalina Lighting Inc., clip lamp model: 2040, China; Bulbs: red and green 60W incandescent bulbs) were fixed to the wall near the headgate so that they were visible to the heifer, and to the headgate operator. The computer operator controlled the red light, while the headgate operator controlled the green light. Figure 1 provides a schematic of the chute and the placement of lights, cameras, and data logging equipment.

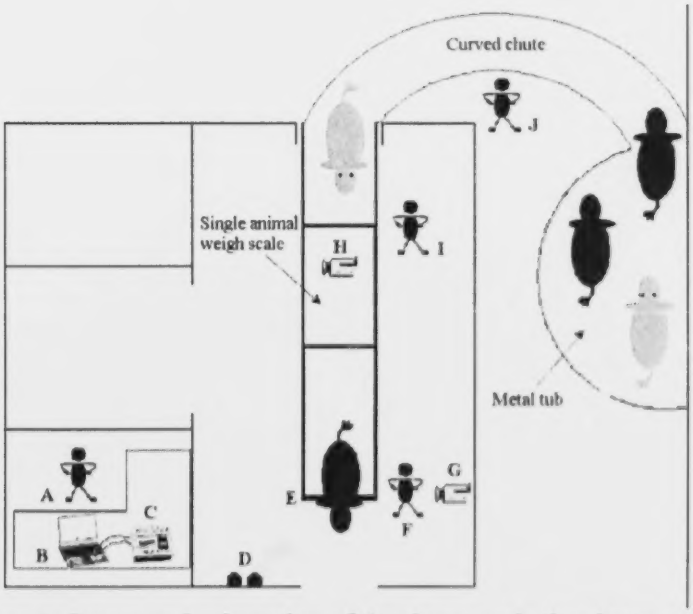

Figure 1: Set up and orientation of the chute, scale, headgate, and experimenters. This diagram is not to scale. Legend: A-Computer operator; B-Laptop computer; C-Datalogger; D-Red and green signal lights; E-Headgate; F-Headgate operator; G-Camera for recording behaviours at the headgate; H-Camera for recording behaviours on the scale; I-Scale operator/Indoor handler; J-Outdoor handler.

2.4 Experimental protocol

After temperament scoring, the heifers were given 4 additional days to acclimatize to their new home pen environment prior to the start of the experiment. Experimental trials were run between 1000 and 1400 hrs on 5 consecutive days. On days 1-4, heifers were moved into the chute and individually held on the scale for 1 minute. They were released from the scale, moved forward and caught in the headgate. The heifers were manipulated for 15 s, by touching their ears, neck, shoulder, rump, and tail, to simulate the human contact that animals would experience during routine procedures. After the touching procedure, the computer operator/time keeper switched on the red light. The headgate

operator then waited until the animal displayed the desired behavioural criterion for its treatment group. Once the animal met its treatment criterion, the headgate operator switched on the green light and released the animal. Exactly when the animal had met its criterion was judged by the headgate operator, rather than the computer operator, in order to minimize reaction time delays, as well as to better simulate what would actually occur during regular on-farm handling.

The protocol for the fifth day followed the same steps up until the point of the illumination of the red light (see Figure 2a & b). On the fifth day, after the red light was switched on, the headgate operator waited an additional 15 s before turning on the green light, regardless of the animal's response. This was done to determine if the animals formed an expectation of the conditions for their release (i.e. had the animals learned which behaviours were required of them for release?). It also gave us a constant time interval for each animal to aid in our final evaluation. After a further 15s, both lights were extinguished and the animal was released.

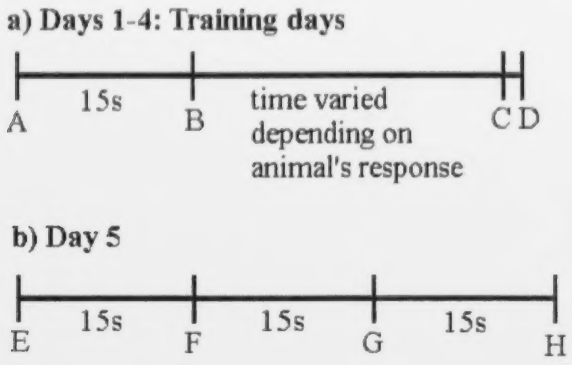

Figure 2: a) Diagram of the procedure used on the training days (days 1-4). A-Animal caught in the headgate; B-The red light is switched on; C-The green light is switched on after the animal has met its treatment criterion; D-Animal is released immediately after the green light is illuminated.
b) Diagram for the Day 5 procedure. E-Animal is caught in the headgate; F-The red light is switched on; G-The green light is switched on (both lights are illuminated at the same time); H-Both lights are extinguished and the animal is released.

During the pre-test temperament scoring and five-day data collection, cattle were moved up through the chute system using a slightly modified version of the protocol outlined in Schwartzkopf-Genswein *et al.* (1997). The handling procedure allowed animals to move forward through the chute complex and up to the headgate on their own accord, but called

for a systematic stepwise increase in pressure, as applied by the handler, if the animal refused to move. This standardized the procedure for ensuring movement of the heifers through the chute system, and was intended to minimize the stress due to handling prior to their being caught in the headgate.

2.5 Data collection

Data from the exertion forces generated on the headgate were collected via the strain gauges and data logger, and recorded on the computer. Videotapes were used to record the time interval between the red and green lights and the point of release from the headgate.

2.6 Statistical analysis

The Statistix software program (Student edition, version 7.0, Analytical Software, 2000) was used to analyze these data. One heifer from each treatment group failed to meet the treatment criteria within three minutes. Data from these two outliers were excluded from the analyses. A two-sample t-test was used to determine the difference in animal weights between the two treatments, and a Pearson correlation was used to evaluate the relationship of animal weight to exertion force on the headgate. Wilcoxon Signed Rank tests were used to determine the significance in the latency to meet criteria for each treatment group. Statistical significance for the exertion force differences over the course of the experiment was evaluated using a Kruskal-Wallis test.

3. Results

The mean weight of animals was not statistically different between treatment groups (Calm=629.3±10.6 kg, Struggle=639.6±10.3 kg, $p=0.88$). Furthermore, there was no correlation between the weight of the animal and the force exerted on the headgate (Pearson correlation coefficient=0.14, $p=0.37$).

Over the course of the experiment, the time to reach criteria significantly decreased for the animals in the Struggle treatment group (D1=12.9±5.3 s, D4=3.8±1.5 s, $p=0.01$; Figure 3). Animals in the Calm treatment group showed no significant change in the amount of time it took them to reach the release criteria (D1=26.3±5.2 s, D4=21.8±5.9 s, $p=0.33$; Figure 3). Figure 3 also shows that the group on the Struggle treatment met the release criteria sooner with each successive training day (days 1- 4). This was not true of animals on the Calm treatment. Over the course of the experiment, both treatment groups exerted significantly less force on the headgate when day 1 is compared to day 5 exertion forces ($D1_{Struggle}$=63.3±8.0 με, $D5_{Struggle}$=43.0±4.6 με, $p=0.02$; $D1_{Calm}$=40.4±3.4 με, $D4_{Calm}$=31.4±2.5 με, $p=0.02$; Figure 4).

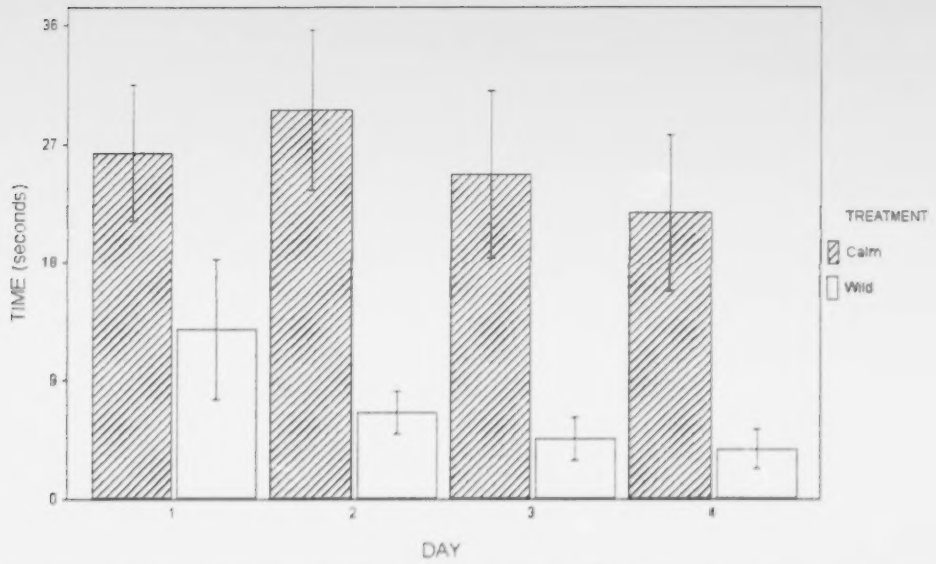

Figure 3: Average time (seconds, s) taken to reach the treatment criteria on subsequent days (day 1-4) for the Calm and Struggle treatments.

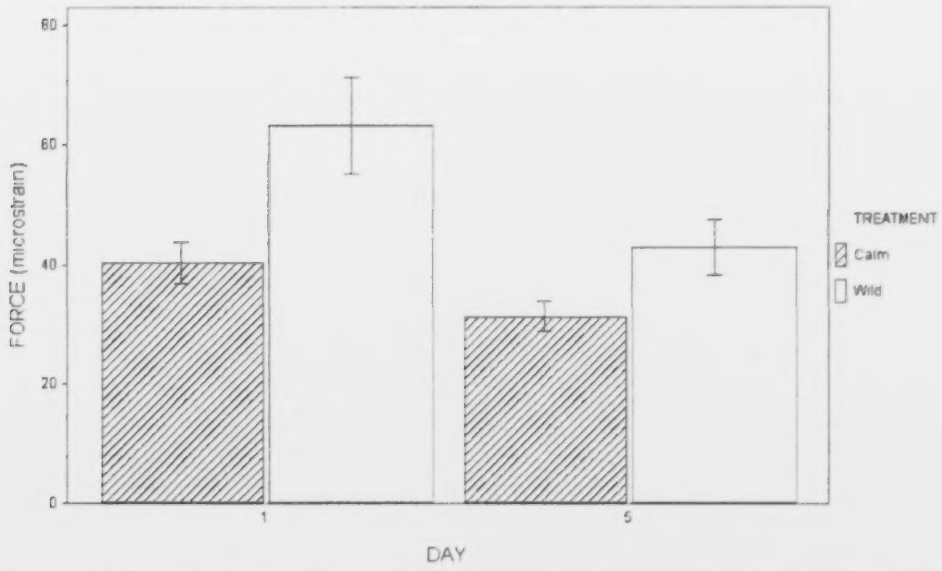

Figure 4: Average exertion forces (microstrain, με) generated on the headgate during the period of restraint on Day 1 compared to Day 5 for the Calm and Struggle treatments.

4. Discussion

The time animals took to meet the release criteria suggest that it is easier for beef cattle to form an association between struggling (doing *something*) to gain release than it is for them to form an association between calmness (doing *nothing*) and release. For a species whose main defensive strategy is flight, standing still is not an intuitive way to gain

release, whereas the tendency to fight against restraint is a more likely innate escape mechanism. The data show that beef cattle can make the association between their actions (especially struggling) and the outcome very quickly, within a matter of a few trials. Thus it is possible that cattle producers may sometimes inadvertently reinforce cattle to struggle during restraint by releasing them while they are struggling. Our trial was conducted over 5 consecutive days during which cattle demonstrated the ability to associate their response with gaining release. However, beef cattle are usually handled infrequently, and it is unclear whether prolonged periods in between handling would diminish their ability to make, or recall, such associations. There is evidence that cattle demonstrate good spatial memory (Bailey and Sims, 1998), memory regarding aversive treatments (Ralphs, 1992; Pajor et al., 2000), and recognition of their human caretakers (Rushen et al., 1998). However, the frequency and number of exposures necessary for cattle to make these associations and demonstrate recall may be critical. Goonewardene and coworkers (2000) found that cattle handled every 10 days, were more difficult to move through a facility compared to cattle that were handled every 20 days, when they both experienced the same aversive treatment, suggesting that frequency of handling is an important variable mediating the memory of handling experiences. Under normal industry practices, with infrequent handling, beef cattle may not demonstrate as strong an association between their struggling and subsequent release as we detected in this experiment.

We expected the heifers on the Struggle treatment to exhibit increasing exertion forces on the headgate. However, over the 5 days of trials, although the heifers did take less and less time to reach the release criterion, they also displayed smaller and smaller exertion forces with each successive day. This could be due to the animals' habituation to the handling facilities and procedures. It is possible that the naïve cattle on day 1 struggled due to fear, but over several days their fear may have diminished while they had made the association between struggling and release. A second plausible explanation is that the cattle were only performing the minimum behaviour required to gain release and nothing more. On the Struggle treatment the cattle were simply asked to struggle, rather than to exert a specific amount of force against the headgate. It could be that they learned that moving backwards and forwards, and mildly struggling was enough to gain release, without having to exert a great deal of force on the headgate. Had we used a criterion of increasing force values as a condition for release, we might have succeeded in training the heifers to push harder against the headgate. However, our goal was not to determine whether cattle could learn to increase their exertion force, but to determine whether cattle made the association between struggling and release, which they clearly did.

There are practical applications for these findings. We showed that cattle released while struggling will more readily express that behaviour upon subsequent restraint. However, if producers wait until the animal is calm before they release it, then the animal is not given the opportunity to form the association between struggling and release. If used consistently, this should result in animals standing more quietly in the headgate and requiring less forceful mechanical restraint. Waiting for a period of calm does not take a prohibitively long time. On average, animals on the Calm treatment took roughly 14 s per animal longer to perform the desired behaviour than their Struggle treatment

counterparts. To handle 100 animals in this manner would require an extra 23 min; perhaps a fair trade if a producer gains calmer cattle as a consequence. It is also possible that as the animals learn the association between calmness and release that the extra time required would decrease with repeated handling.

It has been previously shown that calmer animals tend to have higher growth rates (average daily gains) and yield better quality meat (more tender, less bruising, and decreased instances of dark cutting carcasses; Voisinet et al. 1997ab), though this may be strictly a correlation between genetics that control temperament and genetics that influence performance and have little to do with animals learning to be calm. However, calmer cattle are safer to handle from both the perspective of the handler and of the animal, ultimately resulting in a lower stress and better working environment for both. Cattle that make the association between their actions and an event feel more in control and experience less stress (Hagen and Broom, 2004). It would be interesting to determine if animals that have been trained to meet a behavioural criteria prior to release from a headgate, experience less stress during restraint than animals which have no control over the timing of their release. It would also be interesting to investigate how long cattle can retain these associations and to determine the impact of the time interval between handling sessions on learning.

5. Conclusions

Beef cattle do make an association between their behaviour during restraint and the events that follow, and it is possible to train them to alter their behaviour. Releasing cattle from a headgate while they are struggling will, in effect, teach them to struggle during restraint. It would be prudent for handlers to wait for a period of calm during restraint prior to releasing them. While this is not guaranteed to teach them to be calm during restraint, it will ensure that they are not given the opportunity to make the association between struggling and release.

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